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Incidence and Speciation of Candida Species among Non-gravid young Females in Ilorin, North Central, Nigeria

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ABSTRACT: This study investigated the incidence and speciation of *Candida* species among non-gravid young females, using commercially available chromogenic *Candida* speciation media (CHROM agar) for the identification/speciation of medically important yeast and yeast-like organisms in a routine clinical mycology laboratory. High Vaginal Swabs (HVS) were randomly collected from consenting non-gravid-young females for the study. The participants also completed a structured questionnaire assessing demographic data, symptoms, and risk factors of candidiasis. A total of 120 females between the ages of 17 and 31 years were randomly recruited for the study. Standard microbiological techniques such as Gram's stain, wet mount and culture on Sabouraud Dextrose Agar (SDA) and CHROM agar were used to analyze the swabs. *Candida* species was isolated from 64 of the 120 females, representing 53.3%. The highest incidence rate of 25.0% was recorded in the 23-25 years age group, followed by 12.5% in the 26-28 years while the lowest incidence (1.7%) was observed in the 17-19 years age group. *Candida albicans* (35.0%) was the most common *Candida* species, followed by *C. tropicalis* (8.3%), *C. glabrata* (6.7%) and *C. krusie* (3.3%), whereas, non-Candida infection constituted 46.7% (Negative cultures). Vaginal discharge (85.5%) and itching (52.5%) recorded the high values, with respect to symptoms. The prevalent risk factors associated with vaginal candidiasis in this study were washing of vagina with soap (Vaginal douching) (72.5%) and unprotected casual sex (16.7%). Whitish vaginal discharge (78.3%) was most prevalent followed by creamish vagina discharge (56.7%). In addition to *Candida albicans*, non-albicans *Candida* spp were isolated from HVS specimens; therefore, public health education is vital. CHROM agar is a simple, rapid and inexpensive method with good sensitivity and specificity for identification and speciation of *Candida* species thus, allowing an early and appropriate antifungal therapy. The results of the study will eliminate the ambiguities concerning *Candida* identification in this country and will contribute to better management and proper treatment of patients.

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KEY WORDS: Candidiasis, vulvovaginitis, Incidence, Speciation, CHROM agar, Risk Factors

Candida is an endogenous opportunist which causes secondary infection in individuals with some underlying immune-compromised conditions. It is a normal commensal in the vagina. The prevalence of candidiasis is reported to be twenty- two percent (22%) and this percentage is equals in both adult and adolescent females (Barrousse *et al.*, 2004). It is also estimated that about seventy-five percent (75%) of women will have at least one incidence of candidiasis in their lifetime (Sobel *et al.,* 1998). *Candida albicans* is the most commonly implicated specie in cases of candidiasis. However, other non-albicans species of *Candida,* including *C. glabrata* and *C. tropicalis* have also been implicated and isolated (Enwuru *et al.,* 2008). Candidiasis is often associated with the production of a thick, white/cream/yellow discharge from the vagina tract. This discharge may be watery, often odourless and usually with an accompanying vulvo-vaginal itching and inflammation (Sobel *et al.,* 1998).

Personal-reported history of vaginal candidiasis among females is estimated to be 20% (Foxman, 1990), about 45% is reported in a general population sampling studies (Reed *et al.,* 1989) while approximately 72% are recorded in family practice clinics (Berge *et al.,* 1984). Several hundred million dollars is spent on the diagnosis and treatment of vaginal candidiasis among women aged 15 to 45 years per year (Reed *et al.,* 1989). Antimycotic/antifungal

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drugs sold over the counter (OTC) can alleviate the condition, but interestingly, about 15% to 20% of women usually, experience recurrent *Candida* infection few months after the use of OTC for the second time (Kaufman and Hammill, 1990).

Speciation of microorganisms helps to identify those species/strains which might be intrinsically resistant to some of the antibiotics/antimycotics (Mokaddas *et al.,* 2007). Conventionally, the speciation of *Candida* isolates is done by Gram staining, wet mount, germ tube test, sugar assimilation and sugar fermentation tests, newer methods include CHROM agar, API systems, Vitek 2 ID system and molecular methods (Golia *et al.,* 2013; Mokaddas *et al.,* 2007). Germ tube test is a rapid method to differentiate *C. albicans* and *C. dubliniensis* from other *Candida* spp. For further speciation chlamydospore formation test, sugar fermentation test and sugar assimilation test can be done. But these tests are time consuming and labour intensive. Among the newer tests, CHROM agar is rapid and cost effective as compared to other expensive systems like API systems, Vitek 2 ID system and molecular methods (Pinjon *et al.,* 1998; Odds and Bernnaerts, 1994; Jain *et al.,* 2012).

Vaginal infection due to Candida is very common in teenagers and pubertal females, although most cases are asymptomatic or not reported (Spinillo *et al.,* 1999; Grigirious *et al.,* 2006; Enwuru *et al.,* 2008). This study therefore investigated the incidence of candidiasis, assessed the associated risk factors and speciated *Candida* isolates using CHROM agar.

MATERIALS AND METHODS

Study Area: This study was conducted from July 2015 to September 2015 at the Medical Microbiology Laboratory of the University of Ilorin, Kwara State, Nigeria. Ilorin is the Kwara State capital in Nigeria. The capital city of Ilorin is situated 306km inland from the coastal city of Lagos and 500km from the federal capital, Abuja. Major towns include Offa and Jebba, located on the Niger River. Its geographical coordinates are 8° 30' 0" North, 4° 33' 0" East. The population is estimated to be 2,591,555 (2005 estimate). There are many secondary schools and higher institutions in the state capital, including: the federally owned University of Ilorin, Ilorin, Federal Training Centre, Ilorin, State College of Education, Ilorin, Kwara State Polytechnic, Ilorin, Schools of Nursing and Midwifery, Ilorin, Kwara State University and Al-Hikma University.

Study design, subjects and sample collection: A total number of one hundred and twenty (n=120) consenting non-pregnant-young females, between the ages of 17 and 31 years, were randomly recruited for this study. The screened participants were divided into groups according to their age (17-19, 20-22, 23-25, 26-28, and 29-31) (years). Participants were excluded from the study if they are pregnant or have been receiving antifungal drugs. Participants were pre-educated and advised on how to obtain a High Vaginal Swab devoid of contamination with the vaginal orifice. A structured questionnaire assessing the demographic information, symptoms, and risk factors was also administered. The High Vaginal Swab (HVS) samples were then transported in sterile containers to the Medical Microbiology Laboratory of University of Ilorin for processing.

Ethical consideration: Ethical approval was obtained from the Kwara State Ministry of Health, Ilorin. Written informed consent was obtained from all study subjects/participants. All information about persons screened was kept confidential.

*Laboratory Analyses***:** Each swab was inoculated onto Sabouraud Dextrose Agar (SDA) plates, streaked and incubated aerobically at 37°C for 72 hours, to obtain discrete colonies. Growth was observed after the period of incubation and characteristic colonial morphology of *Candida* were noted. The *Candida* isolates were then sub-cultured on CHROM agar and incubated at 37°C for 24 hours and the species were identified by type and colour of the colonies on CHROM agar media according to the manufacturer's instructions (Table 3).

For Gram staining, smears were made by rolling the swab on clean-grease free-glass slides. The smears were heat fixed by passing over a bursen burner flame for 3-4 times. The films were then flooded with crystal violet for 60 seconds and were washed in clean running tap-water and then flooded with Gram's iodine for 30 seconds. The films were then decolourized with acetone–alcohol and washed immediately with water and counter-stained with safranin for 30 seconds. The films were washed with clean water and allowed to air dry, then examined under the microscope at x100 magnification with oil immersion. Following examination of the stained preparation, the yeast cells were seen as large purple-violet oval cells indicating Gram positive.

For wet preparation, a volume of 200µl of sterile normal saline was placed into the tube containing the swab stick. The bottom of the tube was tapped gently for few seconds. A drop of saline mixture was placed on a clean-grease free-glass slide and covered with a cover slip. The wet preparation was then examined under the microscope using 100 and 400

magnification. Following examination, oval budding cells of *Candida* species were seen and noted.

RESULTS AND DISCUSSION

Table 1 shows the percentage of *Candida* isolated using three different microbiological techniques. The conventional methods of wet mount, Gram staining and culture on SDA were able to detect 60 (50.0%), 62 (51.6%) and 64 (53.3%) yeast/yeast-like organisms, respectively. On SDA, colonies of *Candida* specie were white/cream/yellow coloured, appearing smooth, glabrous and yeast/yeast-like in appearance after 72 hours incubation. Microscopy unveiled typical spherical to sub-spherical budding yeast-like cells or blastoconidia. The result obtained from culture on SDA shows a 53.3% incidence. With respect to the ages of the participants on one side and the fact that this is a non-hospital based study, the high prevalence rate (53.3%) of candidiasis (Table 4) in this study could be linked to vaginal douching (72.5%) and perhaps unprotected casual sex (16.7%), which are predominant risk factors in this study (Table 5). Poor personal, hygiene, poverty, lack of water supply, sharing of panties are probably, additional implicated factors contributing to high incidence of candidiasis. A prevalence of 22% in both adolescent and adults has been reported in the USA (Reed, 1992). A similar result was obtained in a study in Ghana where 21% prevalence was reported (Abruquah, 2012; Feglo and Narkwa, 2012). The high incidence rate (25.0%) recorded in the 23-25 age group in our study agrees with the 25.6% reported by Abruquah (2012) in Ghana among a similar age group. This may suggest a tentative silent *Candida* infection that may become wide spread among females.

The 64 (53.3%) *Candida* spp. isolated from culture on SDA, sub-cultured onto CHROM agar revealed the different species of *Candida* (Table 3). *Candida albicans* (35.0%) was the predominant species isolated. Among the non-albicans *Candida*, *C. tropicalis* (8.3%) was the most common followed by *C. glabrata* (6.7%) and *C. krusie* (3.3%) (Table 2). Non-albicans candida is on the increase, perhaps, due to a decrease in the immune status of the populace. They are more resistant to fluconazole (Spinillo *et al.*, 1999), therefore species level identification has a direct impact on choice of empirical antifungal treatment. Furthermore, geographic variation in the species isolated may vary, which may require the availability of data on the distribution of *Candida species* in different geographic regions. In our study, *C. albicans* predominated (35%). Similar results of *C. albicans* predominance has also been reported (Reed, 1992; Enwuru, *et al.*, 2008). This could be attributed to the fact that *C. albicans* are able to attach easily to the vaginal epithelial cells through their surface mannoprotein. Mannoprotein in the surface of *C. albicans* as well as germ tube formation and mycelium formation facilitates vaginal mucosal invasion. In addition, this may be linked to its virulent factors which include dimorphism and phenotypic switching. *C. albicans* are able to produces protease and phosphatase which enhance its attachment to human epithelium.

Moreover, it can be deduced that the high incidence rate of *C. albicans* may be due to increased physiological changes in estrogen and rich glycogen content of the vaginal mucosa there by providing adequate supply of utilizable sugar that support its proliferation (Rylander *et al.*, 2004; Jain *et al.*, 2012). Perhaps, the reason *C. albicans* is considered a major component of normal vaginal flora. Therefore, under certain favourable conditions such as use of vaginal douching, broad spectrum antibiotics or corticosteroids and other risk factors that increase the incidence of vulvo-vaginal candidiasis, *C. albicans* will proliferate and increase in number, making it a pathogenic/virulent *C. albicans*. There is correlation between microbial number and the onset of pathogenicity. Non-albicans species were observed at a lesser extent than *C. albicans*. These species play an important role in vulvo-vagina candidiasis (Geiger *et al.*, 1995; CDC, 2002; Ringdah, 2000), *C. galabrata* and *C. tropicalis* are not producing mycelia but they produce proteolytic enzymes that help the fungi to adhere to the vaginal epithelial cells (Mokaddas *et al.*, 2007) thus, increasing the incidence of non-albicans candida which has been reported in various studies (Grigirious *et al.*, 2006; Jain *et al.*, 2012; Abraquah, 2012). Among the non-albicans species, *Candida tropicalis* is reported to be the most predominant species (Abraquah, 2012). In this study also, *C. tropicalis* was the most common non-albicans species isolated (Table 2).

Table 4 reveals the percentage of *Candida* isolated among the various age groups and Table 5 presents data on associated risk factor, symptoms and nature of vaginal discharge. The most prevalent associated risk factor in this study is vaginal douching (72.5%) whereas; unprotected sexual activity recorded 16.7% (Table 5). The highest incidence rate of 25.0% was recorded in the 23-25 years group, followed by 12.5% in the 26-28 years group (Table 4). Vaginal discharge (85.8%) is the most prevalent symptom among participants who tested positive for *Candida*, followed by vaginal itching (52.5%) and then vulvo-vaginal irritation and burning sensation ranked equal (36.7%). These finding are corroborated by the reports of

Grigorious *et al*. (2006), which reported vaginal discharge and pruritus/itching as the most common symptom. In a study of 215 women in India, Ahmad and Khan (2009) reported in their study that the predominant symptoms of vaginal *Candida* infection were pruritus/itching with or without vaginal discharge and vaginal erythema/ inflammation.

The results of this study indicates that all the isolates of *Candida* spp grew well on Chrom agar *Candida* differential medium and this is in line with the fact that this medium has good performance, good turnaround time and having sensitivity for the isolation/speciation of *Candida albicans* and non-albicans (Odds and Bernaerts, 1994). *Candida* isolates after 24 hours of incubation on Chrom agar revealed good luxuriant colours (Table 3). This agrees with previous reports that almost all colonies form definite colours on Chrom agar (Odds and Bernaerts, 1994; Mokaddas *et al.*, 2007).

The clinical importance of species-level identification has been recognized as *Candida* species differ in the expression of virulence factors and antifungal susceptibility (Mokaddas *et al.,* 2007; Pinjon *et al.,* 1998). Therefore, Chrom agar is a simple, rapid and inexpensive method with good sensitivity and specificity for identification/speciation of *Candida* species.

Table 1: Percentage of Candida isolates by technique

Technique	***Candida* (%) n=120**
Wet preparation/mount	60 (50.0)
Gram stain	62 (51.6)
Culture	64 (53.3)

Table 2: Percentage occurrence of *Candida* spp. isolated from patients using CHROM agar

***Candida* spp.**	**No. of isolates (%) occurrence**	**Percentage**
C. albicans	42	35.0
C. tropicalis	10	8.3
C. glabrata	8	6.7
C. krusie	4	3.3
Negative cultures	56	46.7
Total	**120**	**100**

Table 3: Colour of Candida Species CHROM agar during spciation

Age (years)	**Females in various ages (%) isolated (%)**	***Candida***
17-19	5 (4.2)	2 (1.7)
20-22	15 (12.5)	7 (5.8)
23-25	60 (50.0)	30(25.0)
26-28	25 (20.8)	15(12.5)
29-31	15 (12.5)	10 (8.3)
Total	**120 (100)**	**64 (53.3)**

Table 4: percentage of Candida Isolated among various ages

Name	**Colour on CHROM agar**
Candida albicans	Light green
Candida krusei	Rose pink
Candida tropicalis	Metallic blue
Candida glabrata	White

Table 5: Percentage distribution of associated risk factors, symptoms of Candidiasis and nature of vaginal discharge among non-pregnant-young females

	Distribution (%) n=120	Positive for *Candida* (%)	Negative for *Candida* (%)
Associated risk factors			
Insertion of herbs/ointment			
Wash vagina with soap	9 (7.5)	4 (44.4)	5 (55.6)
Antibiotic use/misuse	87 (72.5)	34 (39.1)	53 (60.9)
Unprotected Sex	4 (3.3)	3(75.0)	1 (25.0)
	20 (16.7)	9 (45.0)	11 (55.0)
Symptoms of Candidiasis			
Vaginal itching			
	63 (52.5)	23(36.5)	40 (63.5)
Vaginal irritation	44 (36.7)	18 (40.9)	26 (59.0)
Vaginal discharge	103 (85.8)	42 (40.8)	61 (59.2)
Burning sensation	44 (36.7)	18 (40.9)	26 (59.1)
Nature of vaginal discharge			
Whitish	94 (78.3)	37 (39.4)	57 (60.6)
Creamish	68 (56.7)	28 (41.2)	40 (58.8)
Yellowish	29 (24.2)	18 (62.1)	11 (37.9)

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